

***In the Sequence Listing:***

Please delete previous pages 1-10 containing the Sequence Listing and substitute new pages 1-14 containing the Sequence Listing therefore.

**REMARKS**

Claims 1-16 are currently pending. Claim 16 is withdrawn from consideration. Claims 1-15 are rejected. Claim 12 is canceled. Claims 1, 4-8, 11 and 13-16 are amended. New claims 17-21 have been added. Support for new claims 17 and 18 can be found in canceled claim 12. Support for new claims 19-21 can be found throughout the specification as filed. An abstract of the disclosure has been included on a separate sheet. Previous pages 1-10 containing the Sequence Listing have been deleted and new pages 1-14 containing the Sequence Listing substituted therefore. No new matter has been added. Applicants request reconsideration and withdrawal of all the rejections.

**Sequence Letter**

The Office Action states that the sequence of Figure 2 must be assigned a sequence identification number in the brief description of the drawings. The brief description of the drawings at page 10 of the specification has been amended to identify the sequence of Figure 2 as SEQ ID NO. 7.

The Office Action also states that the sequences on page 13 of the specification must be assigned sequence identification numbers. The sequences on page 13, lines 11 and 12 have been assigned SEQ ID NO.: 8 and SEQ ID NO.: 9, respectively.

Applicants note that previous pages 1-10 containing the Sequence Listing have been deleted and new pages 1-14 containing the Sequence Listing substituted therefore. Applicants note that except for SEQ ID NO.: 7, SEQ ID NO.: 8 and SEQ ID NO. 9 which are disclosed by the application as filed, new pages 1-14 containing the Sequence Listing are identical to the Sequence Listing of deleted pages 1-10.

### **Specification**

The Office Action states that an abstract is required. Applicants have attached hereto an abstract of the disclosure as a separate sheet.

### **Priority**

The Office Action states that the application should refer to the priority PCT document upon which it is based. As requested by the Examiner, Applicants have amended page 1 of the specification to indicate that this application is a 371 application of PCT/EP97/04744, which was filed January 9, 1997.

### **Claim Rejections - 35 U.S.C. § 112, second paragraph**

Claims 1, 6, 12 and 13 are rejected as indefinite. The Office Action states that the claim 1 phrase "said pathogen" lacks antecedent basis. Claim 1, line 3 has been amended to recite "said attenuated pathogen" instead of "said pathogen" in order to clarify the present invention.

The Office Action states that the claim 1 phrase "target cell" is unclear. Applicants respectfully urge that one of skill in the art would understand the phrase

"target cell" may refer to a transformed target cell. Nevertheless, in an effort to advance prosecution, claim 1 has been amended to recite a "transformed target cell".

The Office Action states that claim 1 recites a composition that contains a single component, and therefore, does not distinctly claim a pharmaceutical composition. Applicants respectfully disagree. Although the Office Action states that claim 1 is directed to a pharmaceutical composition, claim 1 is actually directed to a recombinant attenuated microbial pathogen. Applicants urge that claim 1 distinctly claims such recombinant attenuated microbial pathogen, and accordingly, the rejection is improper.

The Office Action states that the claim 13 phrase "a pharmaceutically effective amount" is indefinite. In an effort to more clearly set forth the present invention, claim 13 has been amended to recite "a pharmaceutically effective amount for inducing protective immunity".

The Office Action also states that the claim 13 does not recite a step for providing an antigen and therefore recites an incomplete method. In accordance with the Examiner's suggestion, claim 13 has been amended to recite "providing the attenuated pathogen of claim 1 and formulating the attenuated pathogen in a pharmaceutically effective amount for inducing protective immunity with pharmaceutically acceptable diluents . . . ."

The Office Action states that the abbreviations in claim 6 should be clearly defined upon their first appearance in the claims. Claim 6 has been amended to indicate that AlpA and AlpB refer to adherence-associated lipoprotein A and adherence-associated lipoprotein B, respectively.

Finally, the Office Action states that dependent claim 12 recites two different modes of administration which do not further limit claim 11. Applicants respectfully disagree. Applicants respectfully urge that claim 11 can be further defined by the mode of administration. In particular, the mode of administration may determine suitable forms of the composition of claim 11. For example, administration to a mucosal surface might require a composition in a crème or an ointment form while a composition in the form of an aqueous solution would be suitable for administration via the parenteral route. Indeed, Applicants have rewritten claim 12 as new claims 17 and 18 to clarify this aspect of the present invention.

#### **Claim Rejections - 35 U.S.C. § 112, first paragraph**

Claims 1-15 are rejected as not enabled. The Office Action states that the specification does not provide enablement for preventive or therapeutic live vaccines that express any *Helicobacter* antigen. Applicants respectfully disagree. The present invention discloses that the efficiency of *Helicobacter* proteins (urease subunits) known as immunogens can be increased by expression from a heterologous live attenuated bacterium. Any resulting live vaccine can be administered as an oral vaccine giving about 100% protection against *Helicobacter* infections after a single dose application. In contrast, the administration of the antigen in a purified form (without bacterial carrier) is far less effective. Indeed, in an effort to emphasize this increased protection, claims 1, 4-8 and 13-16 have been amended to make it clear that the antigen is an “immunogen”. By the term “immunogen”, Applicants refer to the ability to induce protective immunity against *Helicobacter* infections. In this way it can be assured that the claims are not directed to

any arbitrary non-immunogenic *Helicobacter* antigens or non-immunogenic mimotopes or epitopes thereof. Applicants accordingly urge that the claims are enabled.

### **Claim Rejections - 35 U.S.C. § 102**

Claims 1, 2, 5 and 10 are rejected under 35 U.S.C. § 102(b) as anticipated by Evans et al., J. Bacteriol., Vol. 175(3), 674-683 (1993). The present invention relates to an immunologically protective living vaccine. This vaccine includes a recombinant attenuated microbial pathogen which comprises at least one heterologous nucleic acid molecule and which is given as a living agent. In the case of the claimed invention, the protection or prevention of infection would be against pathogenic *Helicobacter*. Indeed, as discussed above, claim 1 has been amended to make it more clear that the nucleic acid molecule encodes an "immunogen" which is capable of inducing protective immunity, in particular when used in a vaccine. Applicants note that even through protective immunity might be achieved by the application of a subunit vaccine with the simultaneous administration of an adjuvant (see Doré-Davin et al. discussed below), no potent non-toxic adjuvant was known at the time the present application was filed. Furthermore, several oral immunizations are needed to achieve sufficient protection in the animal model with a subunit vaccine, whereas in the present invention only a single dose administration of the live vaccine is needed.

Evans et al. discloses a recombinant bacterium expressing a *Helicobacter* adhesion subunit protein. However, Evans et al. makes no mention of any medical applications of this protein, nor demonstrates that immunization with the adhesion subunit leads to the development of a protective immune response. Rather, it was shown that

antibodies to the adhesion are found in patients, and therefore, it can be concluded that these antibodies are not protective. Evans et al. clearly does not disclose the immunogenic recombinant attenuated microbial pathogens of the present invention.

Claims 1, 2, 5-7 and 10 are rejected under 35 U.S.C. § 102(b) as anticipated by Odenbreit et al., *Molecular Microbiology*, Vol. 20(2), 361-373 (1996). Applicants respectfully disagree. Odenbreit et al. discloses recombinant *E. coli* cells expressing portions of *Helicobacter* antigens. These antigens are disrupted by transposon insertions in the coding region. As in Evans et al., Odenbreit et al. makes no mention of medical applications of these truncated *Helicobacter* antigens, nor were any immunological studies performed. Odenbreit et al. contains no indication that these antigens are capable of inducing a protective immune response. Odenbreit et al. also describes *Helicobacter* cells containing the transposon clones. These cells do not express a heterologous *Helicobacter* adhesion protein. Instead, these cells lack expression of the natural *Helicobacter* adhesion subunit, due to the transposon insertion within the gene. Such results clearly did not anticipate the present invention.

Claims 1, 2, 5 and 10-13 are rejected under 35 U.S.C. § 102(b) as anticipated by Doidge et al. (WO 95/33482) in light of McGhee et al., *Vaccine*, Vol. 10(2), 75-88 (1992). As a preliminary matter, we note that Doidge et al. does not incorporate the McGhee et al. reference. Instead, Doidge et al. merely lists McGhee et al. as well as numerous other articles in a section entitled "References", which is not enough to incorporate by reference. Doidge et al. contains no statement incorporating McGhee et al., and accordingly, McGhee et al. is improperly cited as part of this rejection.

Nevertheless, Applicants address both cited references in an effort to advance prosecution. Doidge et al. proposes that live recombinant bacteria containing expression vectors for *Helicobacter pylori* catalase can be used for the treatment or prevention of *Helicobacter* infections. In particular, bacteria that colonize the gastrointestinal tract (e.g., *Salmonella* as discussed by McGhee et al.) are discussed as suitable for such a purpose.

At the time the present invention was filed it was known that attenuated bacterial carriers could be used for immunizations against bacterial- or virus-associated diseases. Indeed, McGhee et al. postulates that oral immunization preferentially induces type 2 (Th2) cell responses. However, it is state of the art knowledge that live vectors such as *Salmonella* induce cell-mediated immunity responses that largely result from a Th1-type response. In this respect, McGhee et al. comments that "antigens delivered by live vectors such as *Salmonella typhimurium* in the murine system and *Salmonella typhi* in humans must consider T-cell responses induced against a live vector in addition to the inserted recombinant antigen" and furthermore "one must consider an appropriate balance between Th1 and Th2 cells for the induction of antigen-specific IgA responses." However, McGhee et al. provides no indication as to how such an appropriate balance could be obtained. Therefore, at the time the present invention was filed, McGhee et al. was unable to indicate whether oral immunization with a heterologous *Salmonella* live vaccine would be suitable for the prevention or treatment of *Helicobacter* infections. Although Doidge et al. proposes that a recombinant *Helicobacter* live vaccine might be used for the treatment of *Helicobacter* infections, no such evidence was presented. Applicants note that lacking any guidance as to how to proceed, those of skill in the art would not know how to make or use the present invention.

Claims 1, 2, 4, 5, 10 and 12 are rejected under 35 U.S.C. § 102(b) as anticipated by Dore'-Davin et al., Gastroenterology, Vol. 110(4), page A898, col. 1, top abstract (May 1996). Dore'-Davin et al. discloses *E. Coli* expression vectors containing DNA sequences encoding the urease B subunit of *Helicobacter pylori* for protection against *Helicobacter felis*. In Dore-Davin et al., recombinant *E. coli* bacteria expressing fragments of the urease B of *Helicobacter* were used as a source for the production and isolation of these peptides, which were then administered as an oral vaccine with the cholera toxin adjuvant. However, Dore-Davin et al. does not disclose the use of any live vaccine. Nor does Dore-Davin et al. disclose any *Helicobacter* antigen capable of inducing protective immunity. Dore Davin et al. does not contain any data indicating that the application of a live vaccine consisting of the recombinant bacteria expressing the *Helicobacter* antigen induces immunological protection in a host. Therefore, the recited heterologous expression of *Helicobacter* antigens in an attenuated bacterium is not anticipatory of the present invention, in particular the protective immunity aspects of the present invention.

Finally, claims 1, 2, 4, 5 and 10-15 are rejected under 35 U.S.C. § 102(b) as anticipated by Michetti et al. (WO 95/22987). Applicants respectfully disagree. Michetti et al. discloses the administration of a composition comprising *Helicobacter* urease peptides wherein such composition comprises a recombinant live vector which expresses a *Helicobacter* urease peptide. However, Michetti et al. discloses only the use of purified, enzymatically inactive urease with cholera toxin as an adjuvant as a formulation for oral immunization. As discussed above, cholera toxin is highly toxic and not suitable for use in humans. Michetti et al. also discloses that urease as a subunit vaccine has to be given in four oral doses, whereas the *Salmonella* live vaccine of the present invention is

protective after a single dose application. Michetti et al. thus does not disclose the protective *Helicobacter* immunogen of the present invention. Michetti et al. furthermore provides no disclosure as to how a skilled artisan could construct a live vaccine comprising said bacteria or a recombinant vector to express *Helicobacter* urease. Like Doidge et al. discussed above, there is no disclosure as to how such a construct can be designed for the successful use as an oral live vaccine. Michetti et al. clearly does not disclose the present invention.

### **Claim Rejections - 35 U.S.C. § 103**

Claims 1-3 and 4-15 are rejected under 35 U.S.C. § 103(a) as obvious over Michetti in view of Russell et al. (U.S. Patent No. 6,030,624).

As discussed above, Michetti et al. proposes the formulation of genetically engineered attenuated live vector which includes a bacterium expressing *Helicobacter* urease. This bacterium could be *Salmonella typhimurium*, *Salmonella typhi* as well as others.

Russell et al. discloses a method of producing an immune response by oral administration of an attenuated strain of bacteria (e.g., *aroA* and *aroD* mutant *Salmonella typhimurium*) wherein said attenuated bacteria express an antigen of interest as a cholera toxin A2/B chimeric protein.

Applicants note that Russell et al. might suggest to one of skill in the art to express *Helicobacter* antigen-cholera toxin A2/B chimeric proteins in order to induce a humoral immune response upon oral application by teaching the advantages of utilizing such a chimeric protein. A combination of the two references might at most suggest recombinant

host cells for the expression of *Helicobacter* antigens linked to a second heterologous nucleic acid that encodes an cholera toxin A2/B subunit. However, the cited references contain no suggestion or motivation for providing the immunological protection of the present invention. For example, no teaching or suggestion is provided for a protective oral live vaccine consisting of an attenuated bacterial carrier that expresses a *Helicobacter* immunogen on its own (non-chimeric), as is claimed in the present invention. In the present invention, surprisingly, an attenuated bacterial carrier expressing a *Helicobacter* immunogen is sufficient to induce protective immunity of about 100% after a single dose application without use of additional adjuvants. The disclosure of Russell et al. merely provides information regarding humoral responses, and thus contains no disclosure as to whether a CT A2/B chimeric protein expressed in an attenuated bacterial carrier would indeed induce such a high level of protective immunity after a single oral application. Moreover, Russell et al. uses cholera toxin A2/B, which stimulates a humoral immune response, as an adjuvant.

Finally, claims 1-4 and 7-12 are rejected under 35 U.S.C. § 103(a) as obvious over Russell et al. in view of Bukanov et al., *Molecular Microbiology*, Vol. 11(3), 509-523 (1994).

As discussed above, Russell et al. discloses a method of producing an immune response by oral administration of an attenuated strain of bacteria (e.g. *aroA* and *aroD* mutant *Salmonella typhimurium*) wherein said attenuated bacteria expresses an antigen of interest as a cholera toxin A2/B chimeric protein.

Bukanov et al. provide a genetic analysis of a variety of *Helicobacter* genes including virulence factors such as *vacA*, *cagA*, *ureAB*, *ureD* and *ureH*.

The cited references Russell et al. and Bukanov et al. provide no suggestion or motivation regarding the *Helicobacter* immunogen or live vaccine of the present invention. Russell et al. teaches the expression of cholera toxin A2/B as a fusion protein which has immunogenic properties to induce a humoral response. However, Russell et al. does not teach or suggest an attenuated pathogen comprising a *Helicobacter* immunogen which is capable of inducing protective immunity. Nor does Russell et al. teach or suggest *Helicobacter* immunogens which are expressed in an attenuated bacterial carrier without cholera toxin A2/B as a fusion partner as in the present invention. As noted above, such formulations are capable of inducing protective immunity of about 100% after a single dose application. Applicants note that Bukanov et al. fails to cure any of the deficiencies of Russell et al.


Applicants respectfully urge that in light of the discussion above the claimed invention is in condition for allowance and request early notification to that effect.

In the event this paper is not timely filed, applicants hereby petition for an appropriate extension of time. The fee for this extension may be charged to our Deposit Account No. 01-2300, along with any other additional fees which may be required with respect to this paper.

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Respectfully submitted,



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